Review

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Outcomes and Outlooks of Therapeutic Application of Modulators of Endogenous Cannabinoid System in Parkinson's Disease

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Abstract: Recent studies have shown that the endogenous cannabinoid system (ECS) of the brain is essentially involved in the pathogenesis of Parkinson's disease (PD), influencing its symptoms by regulating the level of endogenous cannabinoids and altering the activation of cannabinoid receptors (CBR). Therefore, modulation of ECS with new drugs developed for this purpose may prove to be a promising strategy in the treatment of PD. However, fine regulation of ECS is quite a challenge due to the functional diversity of CBR in the basal ganglia. Our review analyses the effects of ECS modulators on experimental PD models and in patients with PD, as well as presents the outlooks for the development of new cannabinoid drugs for the treatment of motor and non-motor symptoms in PD.

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1. Introduction

Cannabis is a genus of herbaceous plants in the cannabis family (Cannabaceae). The history of the therapeutic use of cannabis goes back a long way. 5,000 years ago in China, it was used as a remedy for malaria, constipation, rheumatic pains, and mixed with wine, cannabis served as an analgesic for surgical manipulations. The therapeutic effects of cannabis use as an anticonvulsant, muscle relaxant, antispasmodic and hypnotic remedies are known.

The use of cannabis for medical purposes began to rapidly expand in the first half of the 19th century, when drugs based on it became "over-the-counter" in England, and in 1854 they were included in the US Pharmacopoeia. Cannabis has been recognized as effective in senile insomnia, neuralgia, migraines, gouty pains, clonic convulsions and epileptiform seizures in brain lesions, and, with careful use, cannabis was considered one of the most valuable medicines.

At the beginning of the twentieth century, the use of cannabis in medicine declined dramatically due to the variability in the activity of herbal medicines and their instability during storage, the appearance of unpredictable effects when ingested, the growing popularity of parenteral remedy, the emergence of effective alternative drugs of synthetic origin, commercial pressure, as well as concerns about the use of cannabis as a drug. In 1928, after the ratification of the Geneva Convention and other legislative acts, cannabis was outlawed in many countries.

More than 400 chemicals have been identified in cannabis, but its main active ingredients are cannabinoids, substances that are classified as aryl–substituted meroterpenes. Cannabinoids are found in the stems, leaves, flowers and seeds of cannabis, as well as in the resin secreted by female plants. More than 60 cannabinoids are known, but the most studied are cannabinol (CBN), cannabidiol (CBD) and the main psychoactive component of cannabis, Δ-9-tetrahydrocannabinol (THC) [1]. Cannabinol and cannabidiol are not psychoactive substances and have an additive, synergistic or antagonistic effect on the effects of THC. The cellular targets of THC are cannabinoid receptors of the 1st and 2nd types (CBR1 and CBR2) [2].

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Intensive research on the structure and properties of natural cannabinoids in parallel with the development of synthetic compounds with high activity and stereoselectivity has revealed the main physiological functions modulated by this class of substances. The discovery of CBR and the development of highly selective powerful cannabimimetics contributed to the identification of a family of lipid transmitters serving as endogenous ligands of CBR – endocannabinoids, arachidonic acid derivatives. The main endocannabinoids include: arachidonoylethanolamide (arachidonoylethanolamide/AEA), or anandamide (from Sanskrit ananda — bliss), which was discovered in 1992, and 2-arachidonoylglycerol (2-arachidonoylglycerol/2-AG), identified in 1995. It was found that, according to their physiological properties, endocannabinoids are very they are similar to synthetic cannabimimetics. The subsequent description of the complex biochemical pathways of synthesis, release, transport and degradation of endocannabinoids completed the formation of ideas about a new signaling system, which was named the endogenous cannabinoid system [2].

Currently, it is generally recognized that the endocannabinoid system (ECS) is a universal lipid signaling system that arose in the early stages of evolution and performs important regulatory functions in the body of all vertebrates. According to the latest results of physiological, pharmacological and morphological studies, the main neurophysiological effect of cannabinoids is retrograde regulation of neurotransmitter release by activating presynaptic CBR's located at the axonal terminals. CBR's belong to the family of G-protein-related receptors and are widely represented in many structures of the brain, such as the basal ganglia (including the globus pallidus and the substantia nigra), strsuctures of the limbic and paralimbic systems, cerebellum, cerebral cortex, whose functions are associated with the control of motor activity, cognitive functions, emotional reactions, motivated behavior and homeostasis.

Endocannabinoids are released "on demand" through receptor-mediated cleavage of membrane lipid precursors and serve as retrograde signaling messengers in GABA and glutamatergic synapses, as well as modulators of postsynaptic neurotransmission, interacting with other neurotransmitters. Endocannabinoids are retrograde transported into the presynaptic terminal by a specific capture system and inactivated with the participation of two well–studied enzymes - fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL).

Postsynaptic depolarization causes Ca^{2+} entry through potential-dependent Ca^{2+} channels. An increase in the intracellular Ca^{2+} concentration leads to the synthesis of endocannabinoids in the postsynaptic neuron. Endocannabinoid synthesis can also be stimulated by activation of presynaptic metabotropic receptors, in particular, glutamate (mGlu1/5) and muscarinic (M1/M3) ones. Endocannabinoids are extrude from postsynapse and retrograde activate presynaptic CB1Rs, which, in turn, inactivate Ca^{2+} and activate K^{+} channels through the G-protein, which leads to suppression of neurotransmitter secretion [3].

Recent advances in pharmacology have made it possible to synthesize a large number of compounds targeted at various components of ECS (CBR agonists and antagonists, anandamide capture blockers) and powerful selective inhibitors of endocannabinoid degradation. This allowed to investigate the physiological role of endocannabinoids and opened up new strategies in the treatment of patients with numerous pathological conditions, including neurological diseases, mental disorders and pain [4].

Parkinson's disease (PD) is the second most common neurodegenerative disease (100-250 per 100,000 population), occurring almost everywhere. The number of PD cases increases significantly in older age groups. Thus, in the group over 60 years of age, the disease occurs in 1% of people, and after 75 years of age it occurs with a frequency of up to 3-4% [5]. In 2017, the number of patients in Russia was about 200 thousand people, and at least 20-25 thousand new cases of the disease were registered annually [6]. Modern approaches to the treatment of PD aimed at compensating for dopaminergic deficiency in the nigrostriatal system (levodopa, dopamine receptor agonists) and correcting the imbalance of other neurotransmitter systems of the central nervous system (amantadines, etc.). Although they lead to a distinct symptomatic effect, they are not able to slow down the progression of the neurodegenerative process [7]. In addition, against the background of

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long-term therapy, multiple drug side effects develop, which dramatically reduces the effectiveness of treatment of advanced stages of PD. Existing surgical approaches are also limited only to the effect on individual symptoms of the disease (for example, tremor), but not on its course. Thus, new safe and effective drugs are needed that provide both persistent symptomatic and nosomodifying (neuroprotective) effects in patients with PD [8].

It should be stressed that PD is characterized not only by well-known motor clinical manifestations (hypokinesia, rest tremor, muscle rigidity, postural disorders), but also by a number of non-motor symptoms, such as behavioral disorders in the sleep phase with rapid eye movements, depression, apathy, cognitive decline, impaired sense of smell, pain in muscles and joints, various vegetative pathological phenomena (constipation, orthostatic hypotension, cardiac arrhythmias, salivation and sweating, etc.) [9]. In patients in the advanced stage of PD, non-motor symptoms may even have a greater impact on quality of life than motor disorders [10]. Moreover, traditional antiparkinsonian drugs (levodopa, etc.) are usually ineffective against non-motor symptoms. This is where the potential of new cannabinoid-based drugs may be in demand. It is significant in this regard that polymorphism in the CBR1 gene may affect the risk of one of the key non–motor manifestations of PD, namely, depression [11].

2. The use of ECS modulators in experimental models of Parkinson's disease in vivo and in vitro.

It has been shown that the ECS constituents are actively expressed in the basal ganglia, interacting with glutamatergic, GABAergic and dopaminergic neurotransmitter systems, which suggested the presence of a high therapeutic potential of ECS modulators in PD [12,13]. Endocannabinoid receptors are of particular pathogenetic importance in PD [14], since they are localized on striatum neurons together with dopamine D1/D2 receptors [15].

Elevated AEA levels in the striatum were found in rodents with a 6-hydroxydopamine PD model [16]. Increasing the level of AEA by inhibiting the enzyme of its hydrolysis (FAAH), prevented the death of dopaminergic neurons induced by the neurotoxin MPTP, and also prevented the development of parkinsonian motor disorders in animals [17]. Elevated AEA levels, induced by inhibition of FAAH, activated dopamine synthesis and attenuated the severity of dyskinesia by activating CBR1 in rats with a PD model [18]. Similarly, chronic inhibition of the 2–AG hydrolysis enzyme (MAGL) increased 2-AG level, preventing motor disorders and protecting the nigrostriar pathway from damage in mice with an MPTP-induced PD model [19].

Experimental studies of the effects of natural cannabinoids in parkinsonism models have shown that THC protects cultured neurons treated with MPTP and prevents the loss of dopaminergic neurons during neurodegeneration induced by 6-hydroxydophamine [20]. The positive effects of THC were simulated by activation of CB1Rs by their selective agonist, arachidonyl-2'-chloroethylamide (ACEA) [21]. Δ9-tetrahydrocannabivarin (Δ9-THCV), a phytocannabinoid with antioxidant properties, reduced 6-hydroxydophamine-induced motor deficiency and loss of dopaminergic neurons in the compact part of the substantia nigra of rats and mice with a PD model [22]. Similarly, in the rat PD model, the phytocannabinoid β-caryophyllene prevented gliosis, oxidative stress and death of nigrostriate dopaminergic neurons induced by rotenone [23], and VCE-003.2, an aminoquinone derivative of the non-psychotropic phytocannabinoid cannabigerol, reduced the intensity of the inflammatory process in brain tissue and improved behavioral results in preclinical animal testing [24]. In a preclinical model of PD in rats, CBD had an antinociceptive effect, while the CB1R inverse agonist (AM251) prevented this effect [25]. In striate microglial cells of animals with experimental models of PD, CB2R expression was significantly increased (compared with the control) [26].

Since mitochondrial dysfunction and oxidative stress are believed to play a key role in the pathogenesis of PD and other chronic neurodegenerative diseases, antioxidant phytocannabinoids are promising neuroprotective compounds [27–32].

The effect of marijuana on cognitive impairment caused by 6-OHDA and on the expression of dopamine and cannabinoid receptors in the rat hippocampus was studied in tests with Morris water maze (VLM) and recognition of new objects [33]. The expression levels of cannabinoid receptors and

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dopamine receptors D1R and D2R in the hippocampus were assessed using real-time PCR. It was found that marijuana in behavioral tests improves spatial learning and prevents memory disorders caused by 6-OHDA. In addition, marijuana consumption increased levels of D1R and CB2R mRNA in the hippocampus, which were reduced under the influence of 6-OHDA. Thus, marijuana may have a positive effect on learning and memory disorders, as well as the expression of dopamine and cannabinoid receptors in PD patients.

In one of the papers [34], essential therapeutic mixtures of cannabis components were proposed, suitable for screening medicinal compounds in PD models. A reductionist approach has been applied to determine the minimum necessary mixtures of these components that are amenable to pharmacological formulation. Screening of sixty-three variations of the original cannabinoid mixtures revealed the five most effective mixtures, which proved to be particularly attractive for therapeutic use. The results of this work have shown the importance of a reductionist approach to the development of therapeutic agents based on a mixture of cannabis with a controlled ratio of components for the treatment of PD.

The results of an active search for evidence of the therapeutic effect of phytocanabinoids on preclinical models of PD in vivo were reflected in one of the recent reviews [35], created on the basis of databases of literature MEDLINE, EMBASE, PsychINFO, PubMed and Web of Science core collection. It has been shown that in most studies on rodents with the BP model, the use of phytocanabiroids leads to a significant improvement in motor function and reduces the loss of dopaminergic neurons, with the inclusion of antioxidant, anti-inflammatory and anti-apoptotic mechanisms. The high neuroprotective potential of cannabinoids in in vivo PD models is emphasized, which creates the basis for clinical trials of their therapeutic properties and use as a preventive agent to reduce the risk of PD development.

A recent paper by other authors [36] presents the results of a search for controlled comparative studies that evaluated the effect of cannabinoids or blockers of their transport on behavioral tests in animals with a PD model. The data from the meta-analysis of behavioral tests indicate the relief of motor symptoms and, thus, indicate the feasibility of cannabis testing in the clinic.

In recent years, in in vitro and in vivo experiments related to the use of cannabinoids in PD and other chronic neurodegenerative diseases, cannabidiol (CBD) has been widely used [37–42]. This cannabinoid does not have a pronounced psychotropic effect and its proportion in cannabis plant extract can reach 40% [43].

In one of the experimental preclinical studies, the effect of CBD on nociceptive reactions of mice with a 6-hydroxydophamine-induced PD model was studied [25]. Under the CBD exposure, the nociceptive pain threshold, reduced in these animals, increased significantly. The 3-hydroxyquinone derivative of CBD, VCE-004.8, prevented the death of TH-positive neurons in the substantia nigra in mice with the 6-OHDA model of PD, in parallel with changes in the reactivity of astro- and microglia [44]. In the same work, the cytoprotective effect of VCE-004.8 was confirmed in in vitro experiments on SH-SY5Y neuroblastoma cell cultures exposed to 6-OHDA. Cell survival analysis showed that this effect of VCE-004.8 is mediated mainly by PPAR receptors (peroxisome proliferator-activated receptors), but not CBR2, since it was eliminated by an antagonist of the first (T0070907), but not the second (SR144528) receptor. In another study on cultures of the same cell line [45] with a PD model induced by MPTP, it was shown that CBD interferes with apoptosis by reducing Bax and caspase 3 levels, as well as the content of PARP-1 in the nucleus. The authors believe that the protective effects of CBD may be mediated by activation of the AKT/mTOR pathway, since the mTOR inhibitor rapamycin eliminated the protective effects of CBD.

Recently, the results of a comprehensive study of the CBD effects on behavioral and biochemical parameters in mice with MPTP induced PD model have been published [46]. Authors showed that CBD inhibits cognitive dysfunction and promotes the preservation of spontaneous movements, increases the levels of 5-HT, DA and IL-10 in brain tissue, which is accompanied by a decrease in TNF-α, IL-1β and IL-6, enhances the expression of tyrosine hydroxylase and Bcl-2, reduces the levels of Bax and caspase-3, and also suppresses the expression of the inflammasome pathway mediated by NLRP3/caspase-1/IL-1ß.

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In experiments on mice with a 6-OHDA model of PD [47], the ability of CBD to inhibit the activity of glycogen synthase-3β kinase (GSK-3β), the main inhibitor of the WNT/β-catenin signaling pathway, controlling oxidative stress and inflammation which are the most important pathogenetic factors of PD [48,49]. In experiments on transgenic mice with a PD model, it was shown that under the CBD exposure, motor deficiency is optimized and motor coordination is improved in a modified forced swimming test, as well as biochemical parameters of biosynthesis of fatty acids, arginine, β-alanine, pantothenate/KoA [50]. In in vitro experiments, CBD had a neuroprotective effect on MPTP toxicity, restoring the expression of axonal and synaptic proteins and reducing microglial activation [51].

3. Modulators of ECS in clinical studies of patients with Parkinson's disease.

The results of preclinical and clinical studies have shown that CBR1 in PD modulate motor symptoms and activity of cognitive information processing systems [52,53]. In neurons and astrocytes of the substantia nigra of patients with PD, the expression of CBR2 was significantly increased compared to the control [26,54]. An increase in the level of AEA in the cerebrospinal fluid of patients with PD was found [55,56], indicating a compensatory protective role of AEA. According to the results of positron emission tomography (PET) [57,58], significant regional changes in CBR1 levels are found in patients with PD, unrelated to the severity of levodopa-induced dyskinesia. In PD, low CBR1 levels in the middle superior frontal gyrus are associated with general cognitive disfunction, impaired executive functions and poor episodic memory, and in patients with severe visual-spatial dysfunction, CBR1 content decreases in the precuneus, motor cortex, middle cortex, additional motor cortex, lower orbitofrontal gyrus and thalamus [53]. Synthetic nonselective agonists CBR, HU-210 and WIN55,212-2, protected mouse nigrostrial neurons in an MPTP-induced PD model, probably involving an antioxidant mechanism secondary to CBR2 activation [59,60].

The above indicates that the lesion of the nigrostriatal system is a key event and marker of the development of PD and is associated with changes in the ECS in the basal ganglia, although the mechanisms by which drugs modulating the functional state of CBR affect the

An open pilot study showed that CBD in combination with standard antiparkinsonian therapy minimizes psychotic symptoms typical in the advanced stage of PD, while not affecting cognitive or motor changes in patients with PD [61]. In another double-blind randomized clinical trial involving 21 patients, CBD improved quality of life indicators over 6 weeks of treatment due to its effect on non-motor symptoms of the disease, although the motor functions of patients did not change [62]. Similarly, a pilot study involving 4 PD patients with a sleep disorder with rapid eye movements showed a decrease in the frequency of agitation, kicks and nightmares after treatment based on 99.9% purified CBD [63].

The results of an open observational study involving 22 patients with PD indicate a significant decrease in resting tremor, muscle rigidity and bradykinesia, as well as non-motor symptoms of the disease (sleep disorders, pain syndrome) after smoking marijuana [64]. In addition, during self–assessment via the Internet, 454 identified PD patients who consumed cannabis daily for more than 12 months reported lower disability and fatigue, and almost half of the patients reported a decrease in the consumption of prescribed antiparkinsonian drugs since the beginning of cannabis use [65].

Another study conducted a telephone survey of 47 PD patients who used cannabis in the form of smoking marijuana for an average of 19 months (in Israel, such marijuana use is allowed under strict medical supervision). It turned out that most patient experienced improvements in falls, pain, depression, tremor, muscle rigidity, and sleep at the beginning of drug use [66].

Cannabis extracts improved motor activity and relieved pain symptoms in patients with PD, and also had a dissociative effect on the pain threshold when exposed to high or low temperatures, indicating that peripheral and central nociceptive pathways can be modulated by cannabinoids [67].

One of the most difficult problems in PD is the development of drug dyskinesia, a typical and difficult–to-treat complication of long-term levodopa therapy. After a good response to levodopa in the first few years of its use, it is dyskinesia that makes it difficult to continue levodopa therapy in the advanced stage of PD. There are reports in the literature on the effectiveness of cannabis in

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levodopa-induced dyskinesia in patients with PD [68,69]. However, in randomized double-blind studies, the antidyskinetic effect of cannabis in PD has not been confirmed [70,71]. It can be concluded that to date, the results obtained regarding the antidiskinetic properties of cannabinoids, as well as their effect on other motor manifestations of the disease (tremor, etc.), remain insufficient to judge their effectiveness according to these indications.

The potential benefit of cannabinoids in PD was confirmed by the results of a randomized, double-blind, placebo–controlled study of a synthetic analogue of tetrahydrocannabinol, nabilone - NMS-Nab [72], which included 47 patients with PD who had stable motor manifestations of the disease and pronounced, disabling non-motor manifestations (≥4 points on a non-motor scale MDS-UPDRS-I). At week 4 of the study, the average change in MDS-UPDRS-I was 2.63 (95% confidence interval 1.53-3.74, $p = 0.002$) in the placebo group compared with 1.00 (–0.16-2.16, $p = 0.280$) in the nabilone group (difference: 1.63, $p = 0.030$). I.e., the rate of increase in non-motor disorders in the placebo group was significantly higher than in the nabilone group. There were no serious adverse events against the background of taking nabilone. The resulting significant effect of nabilone on non-motor symptoms in patients with PD was primarily due to its positive effect on anxiety and sleep problems at night. In 77.4% of patients with PD accompanied by sleep disorders, nabilone contributed to their elimination [73].

The use of a tincture containing Δ9-tetrahydrocannabinol and cannabidiol in a 1:1 ratio led to relief of symptoms such as seizures/dystonia, pain, spasticity, lack of appetite, dyskinesia and tremor in patients with PD, and also significantly reduced concomitant use of opioid drugs [74]. An analysis of 13 articles on the use of cannabinoids in PD conducted in one of the papers showed that CBD, THC and nabilone (a derivative of THC) dose-dependently and stably alleviate motor symptoms, and, in addition, THC reduced pain intensity, and CBD improved psychiatric condition [75].

Recently, in Germany, they tried to find out the attitude of patients to the use of medical cannabis (MK) in the treatment of PD [76]. Using a survey based on a questionnaire, general knowledge and interest in MC were assessed, as well as the frequency, methods, effectiveness and portability of its use. In total, 1,348 questionnaires were analyzed. 51% of the participants were aware of the legality of the use of MK, 28% of the different routes of administration and 9% of the difference between delta-9-tetrahydrocannabinol (9-THC) and CBD. The use of cannabis associated with PD was reported by 8.4% of patients, and this was due to a younger age, living in large cities and better knowledge of the legal and clinical aspects of PD. More than 40% of cannabis users reported reduced pain and muscle spasms. Stiffness/akinesia, freezing, tremor, depression, anxiety and restless legs syndrome subjectively improved by more than 20%, and overall tolerability was good. Symptom relief was reported by 54% of users who used CBD orally and 68% who inhaled cannabis fumes containing THC. Compared with CBD intake, it was more often reported that inhaling THC reduces akinesia and stiffness (50.0% vs. 35.4%; $p < 0.05$). 65% of respondents reported their interest in using MK. Thus, MK was considered by many patients with PD as a therapeutic option.

Cannabis treatment of PD patients was used in one of the studies in 5 randomized controlled and 18 non-randomized trials [77]. In most patients, favorable results were noted regarding the relief of tremor, anxiety, pain, improved sleep quality and life in general. The authors emphasized the need for further well-planned randomized trials.

Noteworthy are the results of a randomized, double-blind, placebo-controlled cross-examination of the effect of CBD on anxiety, frequency and amplitude of tremor in the fear of public speaking test in 24 patients with PD [78], with an assessment of heart rate, systemic blood pressure, frequency and amplitude of tremor. It was found that acute administration of CBD weakens the anxiety caused by CVP and reduces the amplitude of tremor in this anxiogenic situation.

4. Conclusions

The above indicates the undoubted therapeutic potential of directional modulation of ECS in PD [79,80]. In recent decades, ECS attracts considerable interest as a potential therapeutic target for numerous pathological conditions of the nervous system. Since PD is clinically a polymorphic disease with a variety of motor and non-motor manifestations, it corresponds to the multidimensional action

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of ECS modulators and is an adequate object for studying the cellular and molecular mechanisms of their action. Experimental and clinical experience of using ECS modulators in modeling and in the clinic of PD and other chronic neurodegenerative diseases creates the basis for further intensive research of the therapeutic potential of medical cannabis. It is necessary to improve complex experimental methods for the study of ECS, increase the reliability of animal models of PD and further optimize the processes of preparation of cannabis drugs used in experiments. The priority directions in solving the issue of the widespread introduction of ECS modulators into clinical practice should be recognized as the determination of the most preferred cannabinoids suitable for practical use, their optimal dosage and routes of administration, frequency of use and symptoms (non-motor vs. motor), in which the use of cannabis is most optimal. In the PD clinic, it is necessary to further study drugs based on cannabinoids in this category of patients, including the choice of indications, determination and standardization of the optimal composition of the drug and the time of analysis.

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